Action of Chronic Irradiation on the Cytogenetic Damage of Human Lymphocyte Culture

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The action of chronic irradiation (dose rate 2.9 Gy/day) on human lymphocyte culture was investigated. Whole blood was irradiated at 37°C. Aliquots (0.2 ml) of whole blood were cultivated by the standard method. A medium containing phytohemagglutinin was added immediately after irradiation. All structural chromosome- and chromatid-type changes were recorded. The experimental data showed that the conditions of irradiation of lymphocytes affected neither the background level of chromosome damage nor their radiosensitivity. The obtained dose–response curve of chromosome aberrations was described by a linear regression, which then became a plateau. There is no statistically significant difference between the results for the low doses (10–50 cGy) of chronic and acute radiation. — *Environ Health Perspect* 105(Suppl 6):1441–1443 (1997)

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Introduction

The problem of the biologic action of low doses of γ-radiation recently has received much attention. There is experimental evidence that the risk factors at low doses of acute γ-irradiation are greater than expected when extrapolating the high-dose data to lower doses. It was shown by Russell (*1*) that the yield of mutations per unit dose in the range of very small doses was greater than in the range of higher doses. Similar data were obtained in experimental studies of cytogenetic damage (*2–4*), transformation (*5*), and cell survival (*6,7*). Under environmental conditions, however, long-term (chronic) exposure to ionizing radiation of low-dose rate often takes place. In areas of a higher radiation exposure an increase in the level of cytogenetic damage was observed, which was most clearly pronounced in peripheral blood lymphocytes of children (*8*). The aim of this study, therefore, was to investigate the effect of chronic low-dose irradiation on chromosome damage in cultured human lymphocytes.

Materials and Methods

Ten essentially healthy males from the research staff at the Institute of Theoretical and Experimental Biophysics in Pushchino, Russia, were recruited as donors (age distribution 22–30 years). Smokers or persons employed in areas where they could be affected by genotoxins were not included in the donor group. All donors lived at home throughout the experiment, held to a traditional diet and lifestyle, and were physically active. Venous blood was collected between 9 and 11 A.M. by venipuncture and transferred to heparinized plastic tubes.

Standard conditions were used for cell cultivation. Blood (0.2 ml) was added to glass prescription bottles containing 3 ml of complete RPMI 1640 medium (Sigma Chemicals, St. Louis, MO) supplemented with 20% fetal calf serum, 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2% phytohemagglutinin (Sigma Chemicals). Blood samples were cultivated in an incubator at 37°C and 5% CO_2 for 51 hr. Two parallel cultures were set up for each point examined. The same group of donors was used in all schemes of the study.

Whole blood (2 ml) in plastic test tubes was irradiated continuously at 37°C with a chronic γ-field for 0.5, 1, and 2 days at a dose rate of 0.61 cGy/hr with cumulative doses of 7, 13.3, and 27 cGy, respectively. In the case of acute irradiation the samples were irradiated in the dose range of 10 to 50 cGy and 2 Gy at a dose rate of 47 cGy/min. Blood samples from each donor were irradiated at each dose simultaneously. The culture medium was added immediately after irradiation. Colcemid (final concentration 0.25 mg/ml) was added at 49 hr—2 hr before fixation. The cells were treated with a 0.075-M KCl hypotonic solution for 15 min at 37°C and fixed in methanol–acetic acid (3:1). The fixed cells were dropped onto wet slides and stained with Giemsa. Cytogenetic analysis was carried out with 50 to 100 cells in metaphase scored from each blood slide. At least four slides were monitored per person. Dicentrics, centric rings, and chromosome and chromatid deletions, including gaps, were recorded. The data were treated statistically and expressed as mean ± standard error.

In one set of experiments the effect of conditions of chronic irradiation on the frequency of spontaneous chromosome aberrations in human lymphocytes was investigated. In this case eight donors were used (two donors were ill).

In the second set of experiments the effect of storage conditions on lymphocyte radiosensitivity was studied. Blood samples stored for different periods of time were irradiated with a single acute dose of 2 Gy. In these experiments blood samples from the same 10 individuals were used at all storage times. However, after 2 days of storage lymphocytes from only five donors were able to divide.

In the third set of experiments the dose dependence of the yield of chromosome aberrations induced by low doses of acute and chronic γ-radiation were studied. Lymphocytes from the same 10 donors were used in these experiments. For acute irradiation cases, the blood samples were irradiated without storage and the cultures set up immediately after exposure. Frequencies of chromosome aberrations for

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Abbreviations used: cGy, centigray; Gy, gray.

each experimental point were computed as an average yield for all individuals minus the frequency in control.

Results and Discussion

First we studied the effect of storing the blood samples at 37°C for 1 and 2 days on the yield of chromosome structural damage in nonirradiated cells. As seen in Table 1, storing blood samples under these conditions did not affect the background level of chromosome damage.

The effect of storage conditions on lymphocyte radiosensitivity was examined by irradiating blood samples with a single 2-Gy dose (Table 2). Data in Table 2 indicate that there is no statistically significant difference between the average yields of damage per cell for the storage times used. Thus, the storage of blood samples at 37°C for 2 days affected neither the background level of chromosome damage nor their radiosensitivity. This suggests that the culture of peripheral blood lymphocytes can be used for studying the effect of chronic irradiation.

It can be seen in Figure 1 that the dose responses observed in the third set of experiments are described by a step function and almost coincide. There is no statistically significant difference between the results for the low doses of chronic and acute radiation in the investigated dose range. At very low doses (0–20 cGy) the dependencies of cytogenetic damage on dose can be fitted by a linear regression. The linear part of the dose–response curves then becomes a plateau. The spectrum and ratio of different types of chromosome aberrations in lymphocytes in chronic and acute low-level exposure experiments were similar.

Data on the induction of cytogenetic damage in human lymphocytes and other objects at low doses are controversial. The effect of low-dose irradiation on the induction of micronuclei has been studied by several researchers since it was reported that micronuclei arise either from acentric fragments or lagging chromosomes (*9*). Fenech and Morley (*10*) found a linear relationship between the dose and the yield of micronuclei in cytokinesis-blocked human lymphocytes. Cole et al. (*11*) found a linear dose dependence of micronucleus induction in mouse erythrocytes. Other authors observed deviations from linearity in cultured human, mouse, and rat peripheral blood lymphocytes (*12–14*) and in mouse bone marrow erythrocytes (*15*).

Table 1. Yield of chromosome aberrations in human lymphocytes from blood samples stored at 37°C for different periods of time.

Period of storage[a]	Donors, no	Metaphases[b]	Total aberrations, no	Chromosome aberrations			Chromosome aberrations[c]
				Exchanges	Deletions Csm	Deletions Ctd	
0	8	324	2	–	1	1	0.006
1	8	263	2	1	–	1	0.007
2	8	282	2	1	1	–	0.007

Abbreviations: Csm, chromosome; Ctd, chromatid. [a]In days. [b]Average number scored per donor. [c]Average yield per cell.

Table 2. Yield of chromosome aberrations in human lymphocytes from blood samples stored at 37°C for different periods of time before acute γ-irradiation (2-Gy doses).

Period of storage[a]	Donors, no	Metaphases[b]	Total aberrations, no	Chromosome aberrations			Chromosome aberrations[c]
				Exchanges	Deletions Csm	Deletions Ctd	
0	10	315	191	126	50	15	0.60
1	10	286	186	86	86	14	0.65
2	5	100	70	40	25	5	0.70

Abbreviations: Csm, chromosome; Ctd, chromatid. [a]In days. [b]Average number scored per donor. [c]Average yield per cell including respective background levels.

Our previous studies (*4*) have shown that the dose–response curves for micronucleus induction in Chinese hamster fibroblasts, root cells, and dry seeds of *Vicia faba* exposed to acute and chronic γ-radiation were also described by a step function. At very low doses the dependence of cytogenetic damage on dose can be fitted by a linear regression, which then becomes a plateau in the case of fibroblasts and root cells in the dose range of 50 to 100 cGy and a dose range of 8 to 32 Gy for dry seeds. The plateau in a radiosensitive system of human lymphocytes was observed at lower doses than in a radioresistent system of Chinese hamster fibroblasts and *Vicia faba* (*4*). We have shown that caffeine, which inhibits the repair of cytogenetic damage, did not increase the number of cells with micronuclei in the first linear region of the dose-dependence curve and did increase it in the dose interval of the plateau (*4,14*). These results suggest involvement of the repair processes in the formation of chromosome aberrations in the dose range corresponding to the plateau. It could be assumed that the initiation of repair occurs only at a definite level of damage and that the increased yield of cytogenetic damage at low radiation doses is attributable to an insignificant contribution or the absence of repair processes. We have also shown that treating Chinese hamster cells with β-mercaptoethylenamine, a well-known radioprotector, results in the extension of the plateau up to 2 Gy. This is evidence that the plateau does exist and our interpretation of the experimental results is correct (*14*).

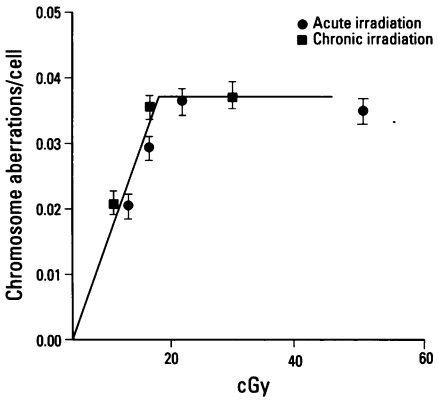

Figure 1. The dose–response curve for chromosome aberration induction in human lymphocytes after acute and chronic γ-irradiation. Values represent means ± standard error. Ten donors per point and 200 to 400 cells per donor were examined. The data indicate the scored yields of chromosome aberrations per cell minus respective background level.

Our investigation indicates, therefore, that it is possible to use peripheral blood lymphocytes to study the effect of chronic low-dose irradiation *in vitro*. The dose-dependence curves of chromosome aberrations in a culture of human lymphocytes exposed to acute and chronic γ-irradiation are described by a step function in the dose range of 0 to 50 Gy and almost coincide.

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